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Summary

Major progress is reported for the period 2-28-09 to 2-28-10. We have successfully achieved the first recordings from any laboratory of the small palisade output neurons from the lobula of *Drosophila melanogaster*, using in vivo targeting of green fluorescent protein expressing neurons. These studies reveal that microneurons with short axons conduct exclusively by graded potentials and that their responses are most likely summed with glomeruli to provide ambiguous detection of defined stimuli. Interpretations of these complex electrotonic responses has lead to novel methods for frequency analysis and data extraction. Further advances have been accomplished on modeling optic glomeruli, using data sets from anatomy and electrophysiology. Pilot studies across insects and reptantian crustaceans reveal optic glomeruli as ubiquitous circuits in the brains of arthropods. Organization clearly reflects visual ecologies and hence behavioral demands. Studies of trace nervous systems in deep time supports theories proposing that glomerular circuits are ubiquitous, ancient, and have evolved to support information extractions from probably all sensory modalities.

1. Recordings from optic glomeruli

Patch clamp recordings from identified microneurons in *Drosophila*: lobula output neurons.

Rationale and strategy. We have invested heavily in setting up a patch clamp recording rig equipped with infrared imaging and computer-driven diode visual stimulation. The rationale for this set up is a long-term effort to use *Drosophila* wild type and, later, mutant flies for investigating the organization of optic glomeruli, with direct comparisons with functional glomeruli of the olfactory system. A first step towards this has been the establishment of a cooperative effort with Dr. Kei Ito, at the University of Tokyo, who has generated many hundreds of wild type lines, in which the expression of a fluorescent green protein has been engineered into specific clones of neurons. This procedure has succeeded in identifying several of the ensembles of neurons that project to between 6 and 8 glomeruli from the lobula. We have crossed the relevant lines supplied by the Ito laboratory to generate animals that reveal cohorts of lobula outputs to identified optic glomeruli.

Using infrared illumination and optics, the cell bodies of such clones are directly observed. A patch clamp recording electrode, filled with biocytin or some other appropriate dye is targeted at and then lowered onto the surface of one of these neurons. Once contiguity between the neuron and electrolyte of the electrode has been established, the cell is recorded during the presentation of a sequence of visual stimuli: stripes, oriented edges, flicker, and others. The responses are gathered digitally and then processed.

Results.

Imaging the neuronal assembly. Figures 1 and 2 show GAL 4 lines. Fig. 1 shows numerous neurons and their cell bodies. These can be selected for probing typical responses. Fig. 2 is a restricted line used for recording from a specific type. Fig. 3 shows two computer reconstructions of recorded short axon and long axon neurons. The left cell is a non-spiking lobula output. The cell to the right is a spiking central body neuron that integrates visual and other modality information.

We have obtained the first publishable results from the first year of experimentation and are now preparing the paper for submission to the Journal of Neuroscience. It was initially a concern that neurons that connect the lobula with optic glomeruli do not spike. The "red flag" was that their electrotonic signals could have been due to decrement of a spiking response due to transmission from the axon to the cell body via a very thin connecting neurite. However, control experiments, recording from neurons with long axons that link the left and right side of the brain, or have other long-distance trajectories, show that such neurons do indeed spike and that these spikes are routinely recordable from the cell bodies. Measurements of the sizes of cell bodies of short-axoned neurons from the lobula to optic glomeruli and cell bodies belonging to long axoned neurons show that these are not statistically different. Likewise, measurements of the diameters and lengths of the neurites connecting cell bodies of these two classes of cells to the integrative parts of the neurons likewise show no significant differences.

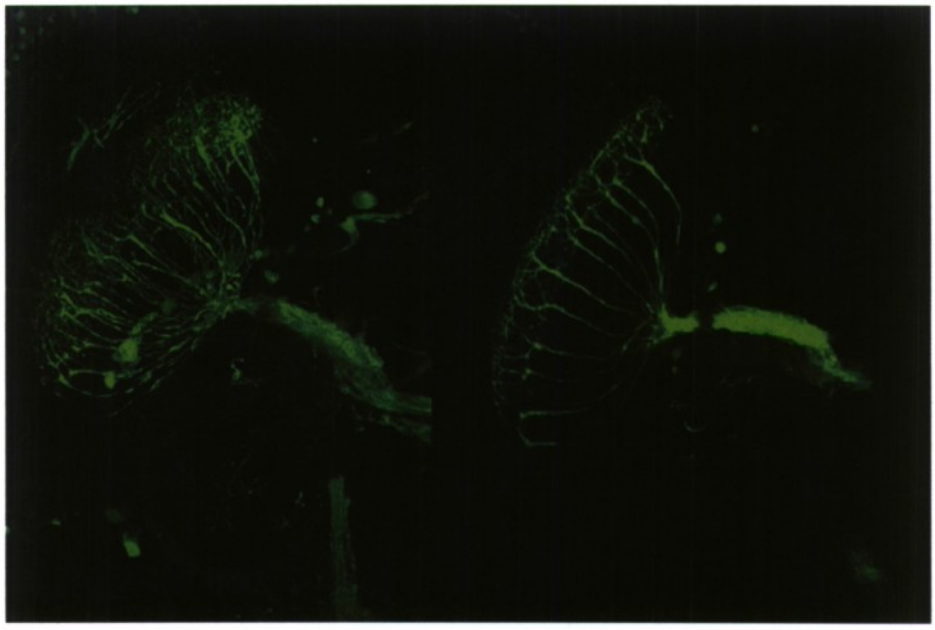

Fig. 1, 2. Gal 4-expressing neurons. Fig. 2 (right) illustrates the line selected for the current study.

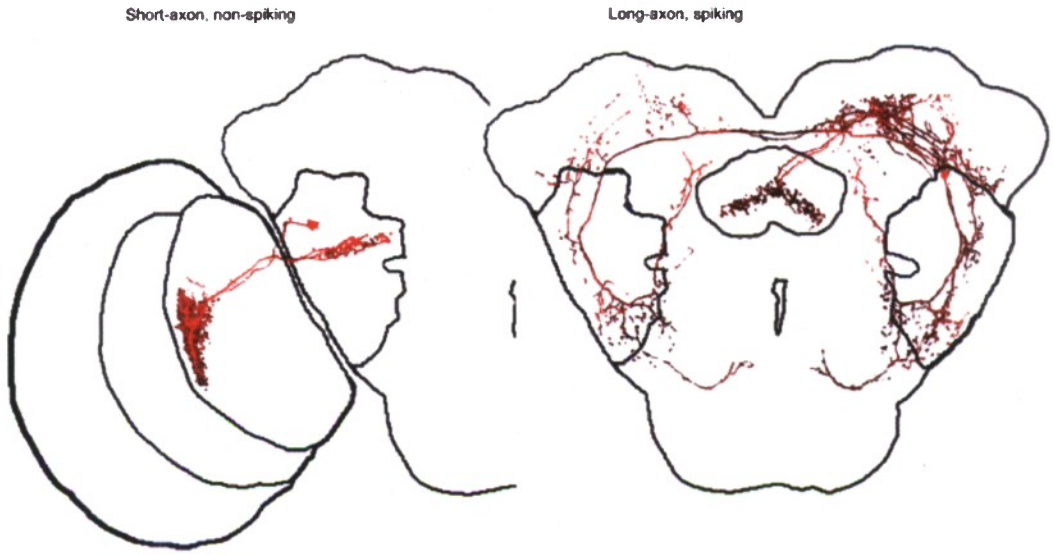

Fig. 3. Reconstructions of non-spiking and spiking interneurons. Width of midbrain is 105 μm.

Recordings. Recordings from the axons of short-axoned neurons in the medullas of larger flies (*Phaenicia serricata*) also showed that these respond by graded potentials. In contrast, recordings from the axons of long-axoned neurons resolved these as spiking neurons. Recordings from anaxonal (cells without axons) local interneurons of the optic glomeruli showed that these also conduct by electrotonic potentials whereas axonal local interneurons in the glomerular complex spike. We thus conclude that the recordings of short-axoned output neurons from the lobula of *Drosophila* are genuine non-spiking neurons.

Non-spiking neurons offer major challenges for data analysis, not only because they is often show no clear and stable resting potential, but also because in the case of these lobula outputs they receive synaptic connections from a variety of sources. Thus, these output neurons can be assumed to be active to some degree irrespective of whether a visual stimulus is presented. Neurons do not simply remain silent until stimulated by the experimenter! As shown in Figure 4, the base line fluctuations can suggest considerable ambiguity with respect to the identification of bone fide responses.

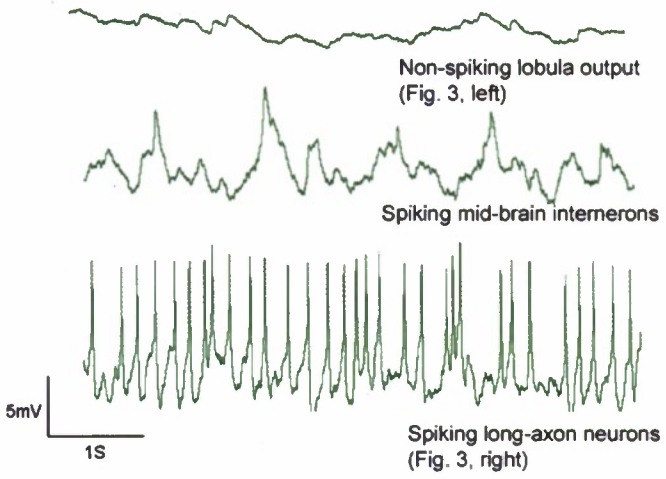

Fig. 4. Response types. The recordings from the examples of neurons are at the same time and amplitude scale. Each represents a different kind of neuron, including non-spiking, spiking and hybrid types. It is known the thickness of the neurite affect the mechanism used by neuron to transfer signal. However, the similar thickness of neurites of three neurons above suggests that it might be the intrinsic function of the neuron itself, which defines the signal transfer mechanism.

The present study shows that any single lobula output neuron is noisy, that it responds unreliably. However, there are about 300 such neurons of the same type, which have the same relationships with retinotopic inputs, that converge to the same glomerulus and hence the same postsynaptic target neurons. Fig. 1, 2 show, from a single section, this principle of retinotopic convergence at a common target centrally.

We predict that the averaged activity of the ensemble will provide reliable coding about visual stimuli. Such convergence is well known at the photoreceptor level of flies, where six receptors that share the same optical alignment and thus "look" at the same point in space, converge to the same postsynaptic target. This convergence is an adaptation for increasing the signal-to-noise ration at low stimulus intensities. We hypothesize that the same principle of convergence and signal extraction operates at deeper levels of the system. It should be recalled that *Drosophila* is minute; its nerve cells are some of the smallest nerve known and they are likely to be individually subject to considerable voltage noise.

Efforts are now being taken to model such an organization and test the above ideas, the hypothesis being that the signals of several lobula outputs together reliably encode the visual response.

Because of the noisiness of single neurons, Laiyong Mu, whose research focuses on this system, as developed methods for data analysis, in which all frequencies of the neuron are visualized and within this spectrum change of frequency can be correlated with the given visual stimulus. Examples are shown in Figures 4, and Figures 5 and 6.

*Time frequency Analysis.*_The analysis was conducted in Matlab 7.9, using program written by the Dr. Mu. Time frequency decomposition was computed through

wavelet analysis, where the recording was convolved with a set of complex Morlet wavelets, defined as a Gaussian-windowed complex sine wave: ei2πtfe-t^2/(2*σ^2). t is time and f is frequency, which ranging from 2 to 80 Hz in 20 logarithmically spaced steps. σ defines the width of each frequency band and was set according to 5/(2πf). 5 represents the number of wavelet cycles and provided a proper balance between time and frequency resolution. After convolution of wavelet, power was defined as the modulus of resulting complex signal Z[t] (power time series: p(t) = real[z(t)2 + imag[z(t)]2]. The baseline was defined as average frequency power from 1s prior to the beginning of each stimulus. The final power time sequences were normalized to a decibel (dB) scale (10*log10[response/baseline]), which allows a direct comparison across frequency bands.

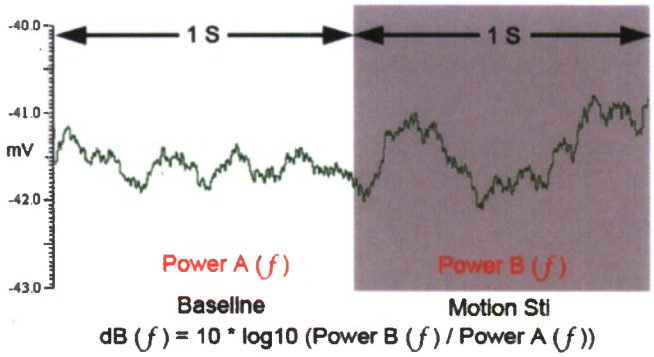

Fig. 5. Raw data and Method Calculation

So far, 5 lobula output neurons have been analyzed for their responses to flicker, light on-off, responses to grid motion in eight orientations, and to edge motion. These results were presented at the Society for Neuroscience Meeting in Chicago, November, 2009.

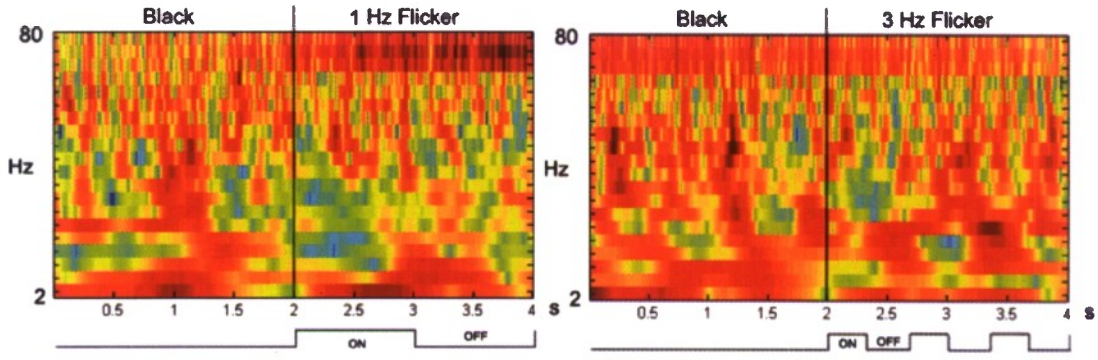

Fig. 6. Left: Time frequency analysis of cell 1112B response to 1hz flicker. The power of 50 to 80 Hz increased when giving 1 Hz flicker, while the power in lower frequency band (3-10 Hz) decreased at the beginning of the flicker.
Right: Time frequency analysis of cell 1112B response to 3hz flicker. The power of 50 to 80 Hz slightly decreased when giving 3 Hz flicker. Additionally, the power in lower frequency band (3-40 Hz) also decreased at during the flicker.

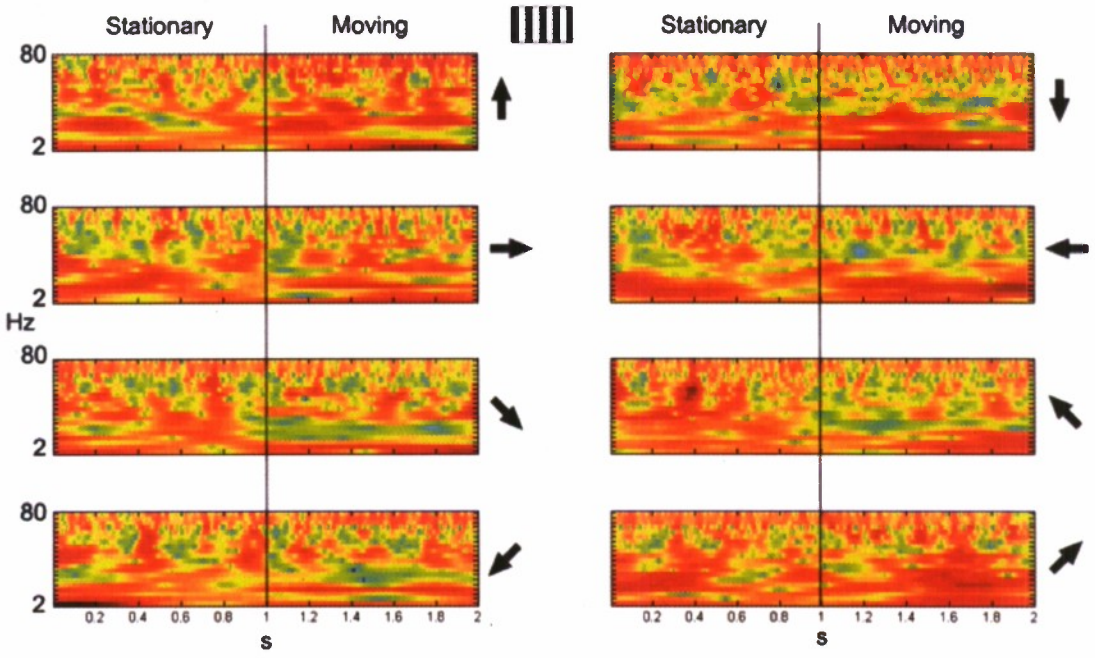

Fig. 7. Time frequency analysis of cell 1112B response to square grating motion in 8 directions This cell showed different responses to the different direction of square grating motion stimuli. For example, there was an obvious increase for the power of 3-10 Hz band in the downward motion. It suggests that this cell has slight preferences for certain directional motion at least in certain trials.

Mu LY, Strausfeld NJ. 2009. Lobula columnar neurons in Drosophila melanogaster and functional relationships to optic glomeruli. Society for Neuroscience. Chicago, IL: Society for Neuroscience, 2009. Online. 850.2/U31.

Mu LY, Strausfeld NJ. 2010. Visual responses to defined stimuli by microneurons: retintotopic lobula outputs from the optic lobes of Drosophila melanogaster. *In preparation* for J. Neurosci.

Structural studies.

Rationale. A fundamental question driving this research is whether the optic glomeruli are representative of a universal integrating network that evidences a ground-plan synaptic organization that serve to decode and encode inputs from any sensory modality. A related question is, therefore, that would this the case, then optic glomeruli should be universal, at least across Arthropoda and olfactory and mechanosensory glomeruli should likewise be universal.

Strategies and preliminary results. Towards this end, we have studied visual neuropils in a group of arthropods that live in a visual object-rich and cluttered environment, do not fly but use active explorative vision. These are the reptantian Eumalacostraca, crustaceans such as crayfish and crabs. Pilot studies begun during the last funding period, using computer-assisted microscopy to obtain very large data sets (at the Max Planck Institute in Jena, Germany) have determined that optic glomeruli characterize these systems too. Although it will be required to expand this study, the first data suggest that the number of glomeruli probably reflect the

complexity of the visual ecology as revealed by elements of that ecology that elicit visual exploration. An important group on which to expand these studies will be arthropods that use visual signaling, such a stomatopods and fiddler crabs.

Comparisons across Insecta also reveal something very interesting: namely, insects that rely on relatively few visual cues for visual choice have fewer glomeruli whereas those that use a variety of ecological signals have more. Thus, honey bee glomerular complexes are less elaborate and have fewer glomeruli than those of *Phaenicia serricata* or those of odonates (dragonflies). Our comparative exploration has also included studies of visually adept arachnids. These also have distinct glomerular arrangements that receive segregated outputs from the principle eye medullas.

An unusual excursion has been an in-depth analysis of an early crustaceamorph from the Mid-Cambrian, which betrays traces of central nervous system, including quite prominent eyestalk neuropil. Although single regions cannot be discerned, the presence of an already substantial lateral protocerebrum and a developed compound eye suggests that sophisticated circuits were already present serving the eye, and that these were probably part of a serially iterated system of glomeruli. Studies of lobopodian fossil material supports this notion. While not a main thrust of this research project, these excursions into deep time provide much food for thought wit regard to the early evolution of glomerular domains and its circuitry. These considerations are currently been written about in the final chapter of a book on brain evolution by one PI (NJS) of this project.

Strausfeld NJ. 2009. Brain organization and the origin of insects: an assessment. Proc Roy Soc Biol Sci. 276:1929-37.

Strausfeld NJ. 2010. Some observations on the sensory organization of the Crustaceomorph *Waptia fieldensis* (Walcott).Proceedings of the International Conference on the Cambrian Explosion. Palaeontographica Canadiana. In press.

Strausfeld NJ, Hansson B. 2010. Optic glomeruli in reptantian crustaceans: comparisons with insect glomerular organization. *In preparation*, J. Comp. Neurol.

Summary of progress on glomerular circuit modeling (Higgins laboratory).

In the past year, the Higgins laboratory has continued its modeling effort to describe the computational structure of optic glomeruli. Higgins and collaborators have created a number of successful variants of a model originally proposed by Hopfield (1991), which is capable of discriminating multiple objects on the basis of their temporal fluctuations. An initial hypothesis on the organization of optic glomeruli used this model with visual inputs corresponding to multiple moving targets on a computer screen, and the model was able to determine the number of objects present and which features corresponded to which objects. The research is currently pursuing two parallel tracks in the last months of the grant: firstly, to maximize performance and understand the parameter sensitivity of the model, and second to marry the model with the most up-to-date biological details of optic glomeruli. Current experiments are using two to four small moving objects in a two-

dimensional visual field, and the job of the model is to determine the number of objects, and the orientation, motion direction, and color of each object. It is expected to have a conference paper or short journal paper contribution on this subject by summer 2010.

Song M, Higgins CM. 2009. A Neuromorphic Model of Optic Glomeruli. Society for Neuroscience. Chicago, IL: Society for Neuroscience, 2009. Online. 278.16/EE58

Dyhr J, Northcutt B, Higgins CM. 2010. A neural network model of insect optic glomeruli. *In preparation*, Neural Information Processing Systems (NIPS), 2010. Vancouver, Canada 2010.

Dyhr J, Northcutt B, Higgins CM. 2010. "Insect optic glomeruli may correlate temporal fluctuations. *In preparation*. Biological Cybernetics, 2010.